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Abstract: BACKGROUND: By analyzing human immunodeficiency virus type 1 (HIV-1) pol sequences from the Swiss HIV Cohort Study (SHCS), we explored whether the prevalence of non-B subtypes reflects domestic transmission or migration patterns. METHODS: Swiss non-B sequences and sequences collected abroad were pooled to construct maximum likelihood trees, which were analyzed for Swiss-specific subepidemics, (subtrees including 80% Swiss sequences, bootstrap >70%; macroscale analysis) or evidence for domestic transmission (sequence pairs with genetic distance <1.5%, bootstrap 98%; microscale analysis). RESULTS: Of 8287 SHCS participants, 1732 (21%) were infected with non-B subtypes, of which A (n = 328), C (n = 272), CRF01_AE (n = 258), and CRF02_AG (n = 285) were studied further. The macroscale analysis revealed that specific subepidemics. The microscale analysis identified 26 possible transmission pairs: 3 (12%) including only homosexual or fall non-B infections diagnosed in Switzerland, < 25% could be prevented by domestic interventions. Awareness should be B subtypes.

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The Role of Migration and Domestic Transmission in the Spread of HIV-1 Non-B Subtypes in Switzerland

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Background. By analyzing human immunodeficiency virus type 1 (HIV-1) pol sequences from the Swiss HIV Cohort Study (SHCS), we explored whether the prevalence of non-B subtypes reflects domestic transmission or migration patterns.

Methods. Swiss non-B sequences and sequences collected abroad were pooled to construct maximum likelihood trees, which were analyzed for Swiss-specific subepidemics, (subtrees including $\geq 80\%$ Swiss sequences, bootstrap $> 70\%$; macroscale analysis) or evidence for domestic transmission (sequence pairs with genetic distance $< 1.5\%$, bootstrap $\geq 98\%$; microscale analysis).

Results. Of 8287 SHCS participants, 1732 (21%) were infected with non-B subtypes, of which A ($n = 328$), C ($n = 272$), CRF01_AE ($n = 258$), and CRF02_AG ($n = 285$) were studied further. The macroscale analysis revealed that 21% (A), 16% (C), 24% (CRF01_AE), and 28% (CRF02_AG) belonged to Swiss-specific subepidemics. The microscale analysis identified 26 possible transmission pairs: 3 (12%) including only homosexual Swiss men of white ethnicity; 3 (12%) including homosexual white men from Switzerland and partners from foreign countries; and 10 (38%) involving heterosexual white Swiss men and females of different nationality and predominantly nonwhite ethnicity.

Conclusions. Of all non-B infections diagnosed in Switzerland, $< 25\%$ could be prevented by domestic interventions. Awareness should be raised among immigrants and Swiss individuals with partners from high prevalence countries to contain the spread of non-B subtypes.

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Over the last decade, the prevalence of human immunodeficiency virus type 1 (HIV-1) non-B subtypes has increased in Western Europe. For example, in a time series extending from 1996 to 2005, Yerly et al [1] noted a steady increase in non-B subtypes among 822 recently infected individuals in Switzerland. Such increases are commonly attributed to migration, as non-B subtypes are mostly found among people from non-European origin.

While Swiss subtype B epidemics have already been studied extensively [2], the possible role of domestic infections with non-B subtypes is not well investigated. A phylogenetic study from the United Kingdom identified 2 clusters of epidemiologically linked A1 subtype sequences involving men who have sex with men of British nationality [3]. Significant clustering of non-B subtypes

was also observed for HIV-1 infected individuals who have acquired the disease through heterosexual contacts. In the United Kingdom, at least 14% (subtype A) and 6% (subtype C) were found to be included in UK-specific clusters, therefore suggesting ongoing transmissions within the UK [4]. Local infections with non-B subtypes in B-dominated regions have also been identified by phylogenetic linkage of individuals diagnosed with primary HIV-1 infection [5]. For example, by screening recently infected patients Yerly et al [6] identified a subtype CRF11 subepidemic among intravenous drug users in Western Switzerland.

The present analysis aimed to study 3 questions: How abundant are non-B subtypes in Switzerland, do Swiss-specific non-B subepidemics exist (macroscale analysis), and finally, can we find evidence for ongoing non-B transmissions in Switzerland (ie, domestic transmission) by identifying possible transmission pairs (microscale analysis)? In order to address these questions, we applied a molecular phylogeny approach to non-B sequences collected from participants of the Swiss HIV Cohort Study (SHCS).

MATERIALS AND METHODS

Sequences and Patients

The SHCS is a nationwide, prospective, clinic-based cohort study with continuous enrollment and semiannual study visits [7, 8]. The SHCS has been approved by ethical committees of all participating institutions, and written informed consent has been obtained from participants. The SHCS drug resistance database contains all HIV resistance tests performed by the 4 laboratories engaged in HIV resistance testing in Switzerland, stored in a central database developed and hosted by SmartGene (Zug, Switzerland, IDNS version 3.6.3) [9]. Resistance data stem from routine clinical testing (60% of tests) and tests performed retrospectively from frozen repository plasma samples (40% of tests). The retrospective sequencing was systematically performed using for each patient the earliest available plasma sample. All laboratories are performing population-based sequencing of the full protease gene and in minimum codons 28–225 of the reverse transcriptase gene using commercial assays (Viroseq Vs.1 PE Biosystems; Viraseq Vs. 2, Abbott AG; vircoTYPE HIV-1 Assay, Virco Lab) and in-house methods [10]. Subtyping was performed on the contig of the protease and the reverse transcriptase sequence using the REGA 2 System [11]. If this method returned inconclusive results, the analysis was repeated with the Star analyzer (<http://www.vgb.ucl.ac.uk/starn.shtml>) [12].

The full SHCS drug resistance database contains 13 750 sequences from 9593 patients. To study the epidemiology of non-B subtypes in Switzerland, we selected all viral sequences from individuals who were seen at least once in the SHCS between 1 January 1996 (earliest availability of plasma samples for retrospective sequencing) and 30 June 2010 (database closure for

this analysis; see Acknowledgments for Genbank accession numbers). Time trends in distribution of B and non-B viruses were analyzed for a subset of these patients who received their HIV diagnosis between 1 January 1996 and 31 December 2009 (to account for time lags in data reporting) through a positive HIV test.

The phylogenetic analysis presented here focuses on non-B subtypes A and C as well as CRF AE and AG, because only these occur in large enough numbers in Switzerland to perform meaningful analyses (see Results). In order to identify Swiss-specific subepidemics, we pooled the earliest available sequence per SHCS participant with all non-Swiss sequences that were available for the same subtype/CRF from the Los Alamos Sequence database (<http://www.hiv.lanl.gov/>, date of accession: September 2010). The following numbers of sequences were available for the different subtypes/CRF: 2018 (subtype A), 2954 (subtype C), 1455 (CRF01_AE), 1643 (CRF02_AG). To avoid a distortion of our analysis by convergent evolution driven by antiretroviral therapy, we removed all major amino acid positions that are associated with antiretroviral drug resistance according to the International AIDS Society - USA (IAS-USA) guidelines [13] (positions 30, 32, 33, 46, 47, 48, 50, 54, 76, 82, 84, 88, and 90 in the PR and 41, 62, 65, 67, 69, 70, 74, 75, 77, 100, 103, 106, 108, 115, 116, 151, 181, 184, 188, 190, 210, 215, 219, 225, and 236 in the RT).

Demographic (transmission groups, geographical origin within Switzerland, nationality) and administrative data (sampling year for genotypic test, year of patient's enrollment in the cohort, year of first positive HIV test) were obtained from the SHCS database. Transmission groups were categorized into heterosexual transmission, men having sex with men, and intravenous drug users. Other transmission modes (perinatal, transfusion risks) were not considered. Information on patient ethnicity is self-reported according to one of the 5 categories: white, black, Asian, Hispanic, or other. Patients' nationalities were categorized as follows for this analysis: Western Europe, sub-Saharan Africa, and Southeast Asia. Because other regions only occurred in small numbers, they were subsumed in 1 category.

Phylogenetic Methods

The phylogenetic methods are described in detail elsewhere [2]. For each of the considered subtypes/CRF (subtypes A and C, CRF01_AE, and CRF02_AG), we inferred maximum-likelihood trees (+100 bootstrap runs) using the GTR model with Γ -distributed rate heterogeneity implemented in RAXML [14]. These trees were then searched for patterns that indicate Swiss-specific subepidemics, which were defined as subtrees including $\geq 80\%$ Swiss sequences and separated by bootstrap support $> 70\%$ from the main tree (macroscale analysis). In addition, trees were analyzed for evidence of domestic transmission, which was defined as possible transmission chains with non-B virus that involved at least 1 individual of white ethnicity

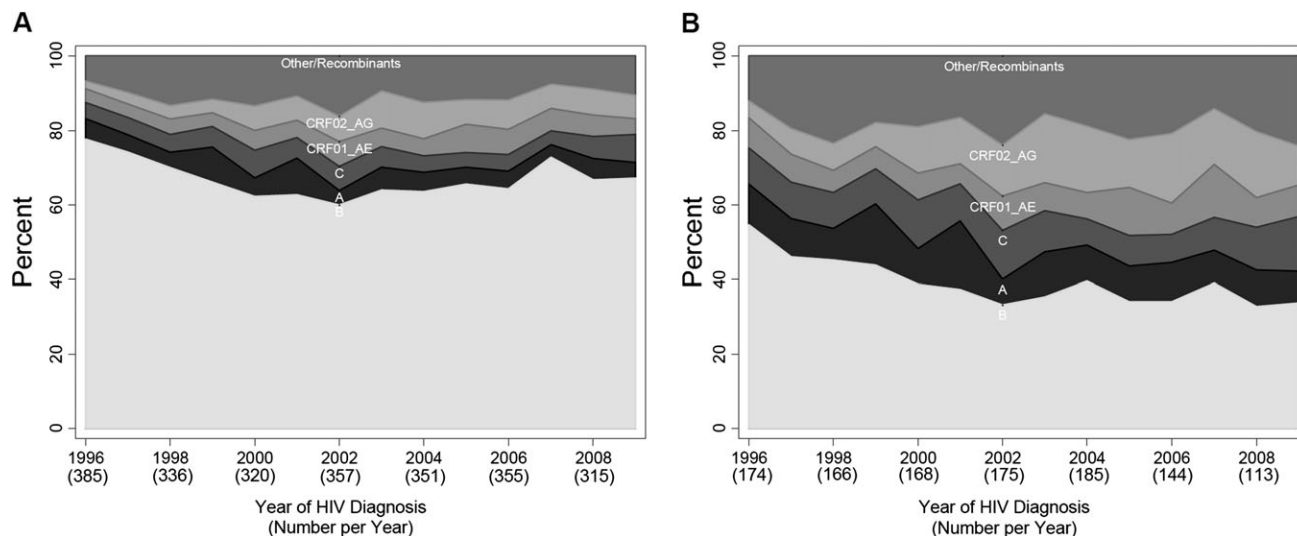


Figure 1. Time trends in distribution of subtypes in the SHCS. *A*, All modes of HIV transmission ($n = 4767$). *B*, Heterosexual contacts only ($n = 2196$). Graphs for intravenous drug users (low numbers) and homosexual contacts only (no discernible time trend) are not shown.

and Swiss nationality (microscale analysis). Possible transmission chains were identified on the basis of sequence pairs with a genetic distance of $<1.5\%$ and bootstrap support $\geq 98\%$.

Statistical Analyses

Categorical data were analyzed with the χ^2 test. Time trends were investigated with the Cochran-Armitage test for trend. Statistical calculations were done with Stata 11.1 (Stata Corp). All P values are 2-sided.

RESULTS

Epidemiology and Time Trends on Non-B Subtypes in Switzerland

Of 10 540 individuals seen at least once in the Swiss HIV Cohort Study between 1996 and 2010, subtype information was available for 8287 individuals (78.6%). Of these 8287 persons, 6555 (79.1%) were infected with subtype B. There were 4 non-B subtypes, which occurred at a prevalence of 3% or greater: A ($n = 328$; 4.0%), C ($n = 272$; 3.3%), CRF01_AE ($n = 258$; 3.1%), and CRF02_AG ($n = 285$; 3.4%). All remaining subtypes contributed $\sim 1\%$ or less to the sequence database; these were D ($n = 55$), F ($n = 74$), G ($n = 99$), H ($n = 8$), J ($n = 4$), K ($n = 2$), CRF03_AB ($n = 2$), CRF06_CPX ($n = 26$), CRF10_CD ($n = 1$), CRF11_CPX ($n = 38$), CRF12_BF ($n = 8$), and CRF13_CPX ($n = 8$). In addition, 267 viral samples (3.2%) were classified as unspecified recombinants of different viral subtypes by the subtyping algorithms used [11, 12].

Time trends in occurrence of B and non-B subtypes were studied for 4767 individuals whose HIV diagnoses (positive HIV tests) fell between 1996 and 2009. Over this time period, the proportion of non-B subtype viruses increased from 22% (86

non-B subtypes/385 HIV diagnoses in 1996) to 33% (78 non-B subtypes/237 HIV diagnoses in 2009) in our study population (Figure 1A; P trend = .004). When analyzing the data for each mode of HIV acquisition separately, there were also appreciable, although in some cases nonlinear time trends for increases in non-B infections among the risk groups of heterosexuals (Figure 1B; total $n = 2196$; 1996: 79 non-B subtypes/174 diagnosed (45%); 2009: 63/95 (66%); $P < .001$), intravenous drug users (total $n = 514$; 1996: 5/85 (6%); 2009: 4/7 (57%); $P < .001$, not shown). In contrast, the proportion of non-B subtypes remained comparably low among 2056 homosexual men (1996: 2/126 (2%); 2009: 11/135 (8%); P trend $< .001$, not shown).

Macrolevel Phylogenetic Analyses Identify Swiss-Specific Non-B Subepidemics

In total, we identified 1143 individuals infected with 1 of the 4 subtypes A, C, CRF01_AE, and CRF02_AG. Characteristics of these patients are displayed in Table 1. Three subtypes (A, C, CRF02_AG) are endemic in sub-Saharan Africa, which is also reflected by the high proportions of individuals with black ethnicity and African origin in these groups (48% in A, 60% in C, 61% in CRF02_AG). In contrast, individuals of Asian ethnicity and origin only contributed 32% to the Southeast-Asia endemic subtype CRF01_AE. Each of the 4 subtype groups also consisted of 24% (C) to 57% (CRF01_AE) individuals of Western European nationality and white ethnicity (other subtypes: 28% in CRF02_AG, 38% in A).

In order to identify non-B subepidemics specific for Switzerland, the phylogenetic maximum likelihood trees constructed on data from the 1143 SHCS participants were searched for subtrees, which were separated from the full tree with a bootstrap support $>70\%$ and contained at least 80% of Swiss sequences

Table 1. Characteristics of SHCS Study Participants Infected With the 4 Most Frequently Observed Non-B Subtypes

	CRF01_AE	CRF02_AG	A	C	P value
N	258 (100)	285 (100)	328 (100)	272 (100)	
Female sex	92 (35.7)	168 (58.9)	213 (64.9)	158 (58.1)	<.001
Ethnicity					
White	158 (61.2)	93 (32.6)	146 (44.5)	77 (28.3)	<.001
Black	2 (0.8)	186 (65.3)	170 (51.8)	180 (66.2)	
Hispanic	2 (0.8)	2 (0.7)	1 (0.3)	8 (2.9)	
Asian	96 (37.2)	4 (1.4)	11 (3.4)	7 (2.6)	
Nationality					
Western Europe	157 (60.9)	88 (30.9)	133 (40.5)	82 (30.1)	<.001
Sub-Saharan Africa	2 (0.8)	174 (61.1)	159 (48.5)	168 (61.8)	
South and East Asia	84 (32.6)	3 (1.1)	10 (3.0)	4 (1.5)	
Other	15 (5.8)	20 (7.0)	26 (7.9)	18 (6.6)	
Study recruitment in German-speaking Switzerland (vs French-speaking) ^a	232 (89.9)	156 (54.7)	235 (71.6)	154 (56.6)	<.001
Mode of HIV acquisition					
Heterosexual contacts	189 (73.3)	269 (94.4)	302 (92.1)	247 (90.8)	<.001
Intravenous drug use	9 (3.5)	2 (0.7)	12 (3.7)	9 (3.3)	
Homosexual contacts	60 (23.3)	14 (4.9)	14 (4.3)	16 (5.9)	
Year of HIV diagnosis (median [interquartile range])	2002 [1998 to 2005]	2003 [2000 to 2005]	1999 [1995 to 2003]	2000 [1997 to 2004]	<.001
Documented seroconversion or HIV diagnosis during primary HIV infection ^b	36 (14.0)	42 (14.7)	33 (10.1)	32 (11.8)	.294
Viral sequence obtained while antiretroviral therapy naive	215 (83.3)	231 (81.1)	206 (62.8)	176 (64.7)	<.001
First measured CD4 count (median [interquartile range])	319 [94 to 488]	284 [168 to 460]	283 [154 to 490]	283 [152 to 510]	.882

NOTE. All numbers are no. (%) unless otherwise stated. Tests of significance for differences across subtypes were performed with the χ^2 -test for categorical variables and the Mann–Whitney *U* test for continuous variables.

^a Individuals from Italian-speaking Switzerland (<4%) were assigned to the German-speaking group due to closer geographical proximity.

^b Negative and positive HIV test <1 year apart or clinical diagnosis of acute retroviral syndrome.

(Figure 2, macroscale analysis). We empirically found that these criteria were well suited to detect Swiss subepidemics because of the low frequency of Swiss sequences relative to the number of added non-B sequences collected outside of Switzerland, which made the risk for falsely detecting Swiss clusters small. Overall, 108 Swiss-specific subepidemics involving 254 (22%) of 1143 non-B infected SHCS participants were detected. Ninety-two of these subepidemics predominantly consisted of persons with a heterosexual orientation (including 206 of the 254 individuals, of whom 6 with a history of intravenous drug use), whereas MSM-dominated clusters were observed in 16 instances (48 individuals). Most of these subepidemics tended to be small, with only 18% of the Swiss specific subtrees consisting of >2 individuals (12% [$n = 13$] and 5% [$n = 5$] with cluster size 3 and 4, respectively, 1 cluster [1%] of size 5, and 1 cluster [1%] of size 14). Although not reaching statistical significance, there was a trend for MSM-dominated subepidemics to form larger transmission chains as illustrated by a somewhat higher fraction of subepidemics reaching a size >2 (25%) compared with heterosexually dominated

subepidemics (17%) and the greater size of the largest cluster (14 vs 5 in heterosexual subepidemics).

As illustrated by Table 2, different patterns of clustering were observed across subtypes. The proportion of non-B infected SHCS participants included in Swiss-specific subepidemics varied significantly (Test for heterogeneity in proportions of clustering across subtypes $P = .008$). The highest degree of clustering was observed for the CRF02_AG group, where 80 (28%) of 285 individuals were contained within 34 Swiss specific subepidemics, followed by CRF01_AE with 61 of 258 SHCS participants (24%, 23 subepidemics). Corresponding proportions of clustering sequences (number of clusters) for subtype A and C were 21% (69 of 328 individuals, 31 clusters) and 16% (44 of 272 individuals, 20 clusters), respectively. Of note, we found no indication for a time trend in these proportions, irrespective of viral subtype (all tests for trend $P > .14$; data not shown). In particular, there was no indication for a higher degree of clustering of viral samples collected in later years, which—if found—could be suggestive for emerging self-sustained non-B epidemics.

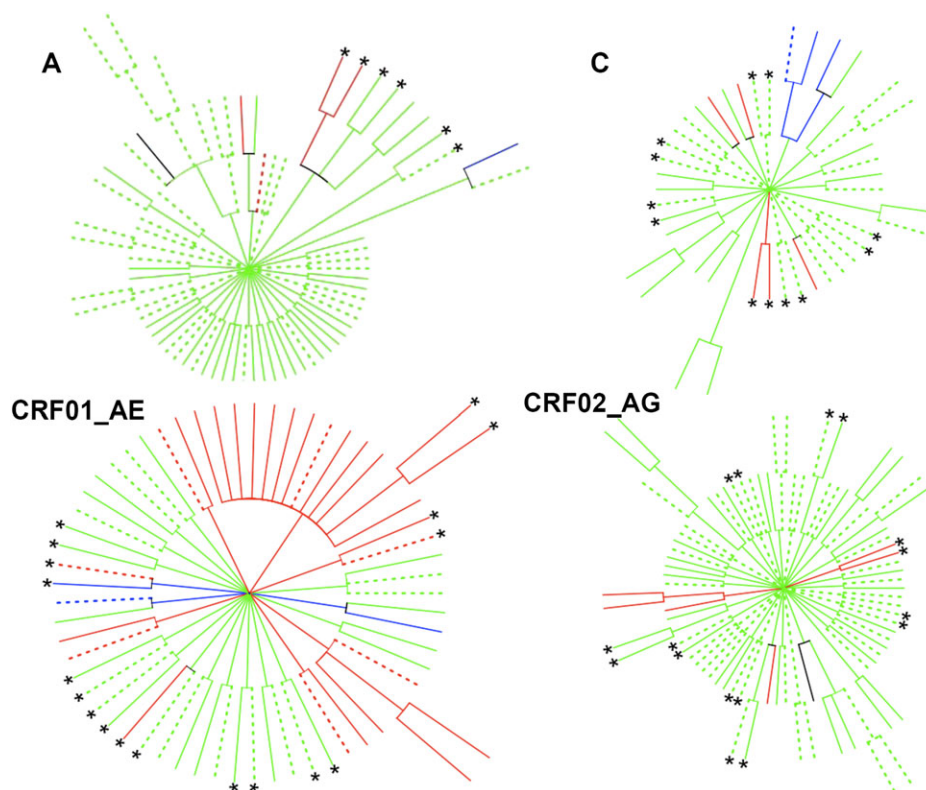


Figure 2. Swiss-specific subepidemics for subtypes A and C and CRFs AE and AG. Only tips belonging to Swiss patients in a Swiss transmission cluster are depicted. Each edge emerging from the center corresponds to one transmission cluster. Colors indicate the transmission group (*green*: heterosexual, *red*: MSM, *blue*: IDU, *turquoise*: unknown transmission group). Dashed lines indicate patients of nonwhite ethnicity. Stars indicate possible transmission pairs

In order to distinguish between possible domestic transmission and immigration, we analyzed clustering patterns according to groups defined by ethnicity and nationality. In particular, we considered sequences contained within Swiss-specific clusters to have likely resulted out of domestic transmission events (ie, to have occurred within Switzerland), and the remaining infections as possibly having been introduced from abroad through migration. Among the African subtypes A, C, and CRF02_AG, the overall proportion of individuals with black ethnicity and Sub-Saharan African nationality included in Swiss-specific sub-epidemics was 16.4% (81/494; subtype A: 31/157 (19.7%), C: 15/164 (9.1%), CRF02_AG: 35/173 (20.2%); test for heterogeneity of proportions across subtypes $P = .0184$), whereas viral sequences from white persons from Western Europe were linked to Swiss subepidemics in 30.2% of cases (81/268; subtype A: 29/123 (23.6%); C: 19/66 (28.8%); CRF02_AG: 33/79 (41.8%); test for heterogeneity of proportions across subtypes $P = .114$). For the Southeast Asia-endemic subtype CRF01_AE the proportion of sequences from Asian individuals contained within Swiss-specific clusters was 22.9% (19/83), and the proportion of clustering sequences among white, Western European individuals was 24.7% (36/146).

Next, we studied sociodemographic factors associated with inclusion of sequences in Swiss-specific subepidemics using

logistic regression. Due to expected differences in sociodemographic factors between the 3 African subtypes A, C, and CRF02_AG and the Southeast Asian subtype CRF01_AE we chose to analyze these 2 subtype groups separately. Univariable logistic regression models restricted to the African subtypes detected associations of male sex (odds ratio 1.8 [95% confidence interval, 1.3–2.4]), white ethnicity (2.3 [1.7–3.3] compared with blacks), Western European nationality (2.1 [1.5–3.0] compared with Sub-Saharan Africa) and homosexual mode of HIV acquisition (2.0 [1.0–2.8] compared with heterosexual infection) with a higher probability for inclusion in Swiss-specific subepidemics. In contrast, the only positively associated factor for the Asian subtype CRF01_AE was HIV acquisition through homosexual contacts (4.6 [2.4–8.8]). Due to the strong cross-correlation of these demographic characteristics, for example between nationality and ethnicity or sex and mode of HIV acquisition, multivariable analyses were not performed.

Instead, characteristics of Swiss-specific subepidemics were further analyzed descriptively and on the basis of demographic subgroups defined according to ethnicity and mode of HIV acquisition. Figure 3A highlights differences in these demographic subgroups between the African subtypes and the Asian subtype CRF01_AE. The major differences found were (1) lower proportions of men who have sex with men in subepidemics of

Table 2. Characterization of Sequences Included in Swiss-Specific Subepidemics

	CRF01_AE			CRF02_AG			A			C		
	Cluster	No cluster	<i>P</i> value	Cluster	No cluster	<i>P</i> value	Cluster	No cluster	<i>P</i> value	Cluster	No cluster	<i>P</i> value
<i>N</i>	61 (100)	197 (100)		80 (100)	205 (100)		69 (100)	259 (100)		44 (100)	228 (100)	
Female sex	16 (26.2)	76 (38.6)	.078	41 (51.3)	127 (62)	.099	39 (56.5)	174 (67.2)	.099	17 (38.6)	141 (61.8)	.004
Ethnicity			.044			.001			.040			<.001
White	40 (65.6)	118 (59.9)		39 (48.8)	54 (26.3)		36 (52.2)	110 (42.5)		24 (54.5)	53 (23.2)	
Black	0 (0)	2 (1)		38 (47.5)	148 (72.2)		32 (46.4)	138 (53.3)		19 (43.2)	161 (70.6)	
Hispanic	2 (3.3)	0 (0)		1 (1.3)	1 (0.5)		1 (1.4)	0 (0)		1 (2.3)	7 (3.1)	
Asian	19 (31.1)	77 (39.1)		2 (2.5)	2 (1)		0 (0)	11 (4.2)		0 (0)	7 (3.1)	
Nationality			.694			.003			.207			<.001
Western Europe	37 (60.7)	120 (60.9)		36 (45)	52 (25.4)		30 (43.5)	103 (39.8)		22 (50)	60 (26.3)	
Sub-Saharan Africa	0 (0)	2 (1)		36 (45)	138 (67.3)		31 (44.9)	128 (49.4)		15 (34.1)	153 (67.1)	
South and East Asia	19 (31.1)	65 (33)		2 (2.5)	1 (0.5)		0 (0)	10 (3.9)		0 (0)	4 (1.8)	
Other	5 (8.2)	10 (5.1)		6 (7.5)	14 (6.8)		8 (11.6)	18 (6.9)		7 (15.9)	11 (4.8)	
Mode of HIV acquisition			<.001			.311			.439			.078
Heterosexual contacts	30 (49.2)	159 (80.7)		74 (92.5)	195 (95.1)		64 (92.8)	238 (91.9)		36 (81.8)	211 (92.5)	
Intravenous drug use	3 (4.9)	6 (3)		0 (0)	2 (1)		1 (1.4)	11 (4.2)		3 (6.8)	6 (2.6)	
Homosexual contacts	28 (45.9)	32 (16.2)		6 (7.5)	8 (3.9)		4 (5.8)	10 (3.9)		5 (11.4)	11 (4.8)	
Study recruitment in German-speaking Switzerland (vs French-speaking) ^a	56 (91.8)	176 (89.3)	.577	42 (52.5)	114 (55.6)	.636	50 (72.5)	185 (71.4)	.865	22 (50)	132 (57.9)	.333
Documented seroconversion or HIV diagnosis during primary HIV infection ^b	14 (23)	22 (11.2)	.020	19 (23.8)	23 (11.2)	.007	8 (11.6)	25 (9.7)	.634	13 (29.5)	19 (8.3)	<.001
Viral sequence obtained while antiretroviral therapy naïve	52 (85.2)	163 (82.7)	.646	69 (86.3)	162 (79)	.162	43 (62.3)	163 (62.9)	.925	32 (72.7)	144 (63.2)	.224
Year of HIV diagnosis	2003 [2001–2006]	2001 [1997–2005]	.010	2003.5 [1999.5–2006]	2003 [2000–2005]	.563	2000 [1996–2003]	1999 [1995–2003]	.591	2002 [1997–2004.5]	2000 [1997–2003.5]	.164

NOTE. All numbers are no. (%) unless otherwise stated. Tests of significance were performed with the χ^2 -test for categorical variables and the Mann–Whitney *U* test for continuous variables.

^a Individuals from Italian-speaking Switzerland (<4%) were assigned to the German-speaking group due to closer geographical proximity.

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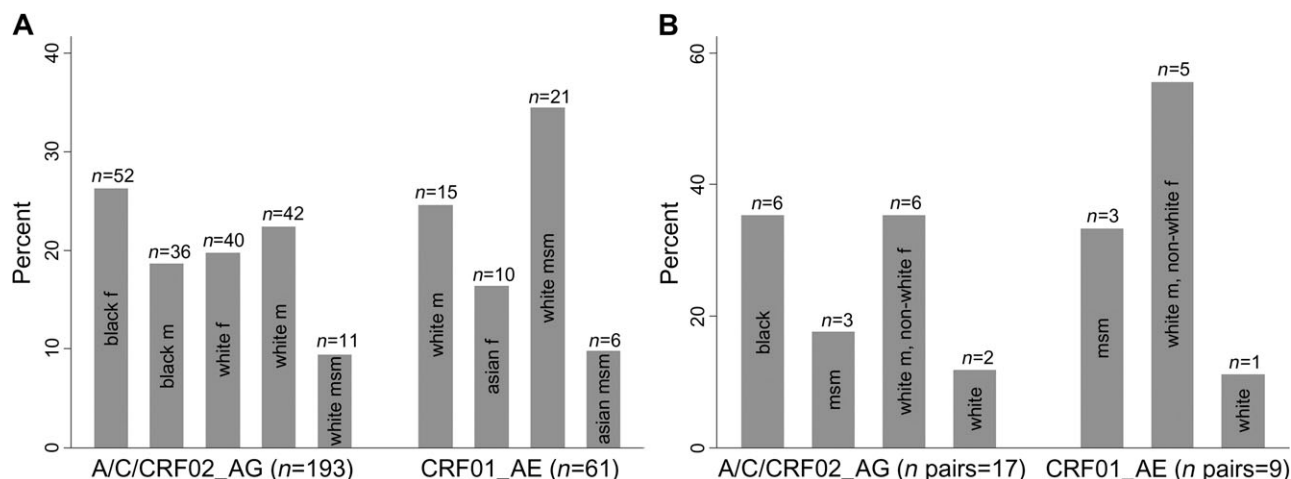


Figure 3. Distribution of demographic groups (defined on sex, ethnicity, and mode of HIV acquisition) among Swiss-specific subepidemics (A, macro-scale analysis) and within possible transmission pairs (B, micro-scale analysis). Note that in panel A only demographic groups with a proportion $\geq 5\%$ are plotted. Mode of HIV acquisition is heterosexual if not mentioned otherwise. f, female; m, male; msm, men who have sex with men.

African subtype when compared with subtype CRF01_AE and (2) the comparably small numbers of white females ($n = 4$) and heterosexual asian males ($n = 1$) observed in CRF01_AE subepidemics (both present at $<5\%$ and therefore not plotted). Conversely, the proportion of white and black men and women appeared to be in balance in clusters of African subtypes.

When performing sensitivity analyses, we found that our conclusions were robust to methodological variations of our approach. For instance, the number of Swiss subepidemics decreases by $<28\%$ on average if the analysis was restricted to subtrees with bootstrap support above 95% (instead of $>70\%$). Likewise, the number of subepidemics changed by $<10\%$ if the fraction of Swiss sequences defining a Swiss subepidemic was increased from 80% to 100%.

Identification of Possible Transmission Pairs in Micro-scale Analysis

In addition to the macro-scale analysis, which identified Swiss-specific non-B subepidemics, we further aimed to detect possible transmission pairs by more conservative criteria (micro-scale analysis), defined as sequence pairs clustering with a bootstrap support $\geq 98\%$ and a genetic distance <0.015 on the trees calculated for the macro-scale analysis [15, 16].

A total of 26 possible transmission pairs were detected (Figure 3B). Among the 17 pairs infected with African subtypes, the most frequently observed types of transmission events were among pairs of heterosexual men and women both of black ethnicity (n total = 6; A: $n = 0$; CRF02_AG: $n = 2$; C: $n = 4$), and heterosexual transmission between individuals of mixed ethnicities (n total = 6; A: $n = 1$; C: $n = 1$, CRF02_AG: $n = 4$). Noteworthy, in all of the latter 6 transmission pairs, the male partner was of white ethnicity. Possible heterosexual transmission between white individuals was observed 2 times

(A: $n = 1$; CRF02_AG: $n = 1$) and possible homosexual transmission between individuals of mixed ethnicity was seen in 3 instances (A: $n = 1$, C: $n = 1$, CRF02_AG: $n = 1$). Among the patients infected with the Asian subtype CRF01_AE, 5 possible transmission events between Asian females and white males were identified, as well as 3 events among white homosexual men. In addition, 1 possible heterosexual transmission event included 2 individuals of white ethnicity.

Overall, when also taking information on nationality into account, local transmission was likely in 16 (62%) of the 26 possible transmission events, because at least 1 partner was of white ethnicity and Swiss nationality. Of 6 possible events involving homosexual transmission, there were 3 pairs of white men from Switzerland and 3 pairs, which included white men from Switzerland and partners from other world regions. Ten possible transmission pairs consisted of heterosexual white Swiss men and females from other world regions; 8 of those were of nonwhite ethnicity. In addition, there were 4 potential heterosexual transmission pairs involving at least 1 white individual from Western European countries other than Switzerland, thus making infection in Switzerland seem less certain.

DISCUSSION

By analyzing >8000 HIV-1 pol sequences from Switzerland, we detected significant increases in the proportion of non-B subtypes over a time period of 12 years, which were most apparent among individuals who acquired HIV through heterosexual contacts. Among the most frequent non-B subtypes with a prevalence $>3\%$ were A, C, CRF01_AE, and CRF02_AG. A macro-scale phylogenetic analysis revealed that only $\sim 20\%$ of newly detected non-B infections among individuals of Asian or African nationality may have originated from domestic

transmission, whereas the remainder of infections most likely have occurred outside of Switzerland. Although this proportion was higher among white, Western European individuals and reaching up to 42% for subtype CRF02_AG, substantial numbers of sequences were not associated with Swiss-specific subepidemics, possibly reflecting infections acquired during travel and stays abroad [17]. Along these lines, the macro-scale analysis further indicated that, consistent with findings from a previous analysis of the Swiss subtype B epidemic among heterosexuals [2], the non-B epidemic can only be maintained through frequent reintroduction from abroad. The presence of self-sustained non-B epidemics would lead to large transmission chains in the phylogenetic tree (similar to those observed among subtype B infected MSM in Switzerland [2]), and possibly also to an increase in the ratio of clustering (ie, Swiss-specific) and nonclustering sequences over time. Neither was seen in our macro-scale analysis.

Our observations from the macro-scale analysis are in line with results from a modeling study, which also found a much greater contribution of migration to newly diagnosed non-B infections among Africans than domestic transmission [18]. The Dutch study predicted a proportion of 60% of newly diagnosed infections among African immigrants to have occurred outside the Netherlands, whereas in our study 80% and more of all non-B infections among Africans may have originated outside of Switzerland, given that only ~20% of all sequences from this group were contained within Swiss-specific clusters.

Nevertheless, our micro-scale analysis yielded substantial evidence for possible domestic non-B transmissions, because, even with a very conservative criterion, we detected 16 instances of genetically linked viral samples including at least 1 individual of white ethnicity and Swiss nationality. A surprisingly large proportion (10/16, 63%) of these possible local transmission events involved a constellation of white Western European males and nonwhite females, which is suggestive for an important role of interracial partnerships in transmission of non-B subtypes within Switzerland. These patterns are also consistent with a contribution of sex tourism or prostitution to non-B infections in Switzerland, as occasionally reported by newly HIV-diagnosed individuals as a possible source of infection [Günthard, unpublished observation].

Several study limitations should be noted. Our analysis is subject to the usual and unavoidable caveats associated with molecular epidemiological analyses such as sampling bias (see Kouyos et al [2] for a detailed discussion), but these problems are substantially alleviated by the high representativeness of the SHCS [8]. Furthermore, the criteria used to identify possible transmission pairs in the micro-scale analysis were strict and have successfully been used previously, confirmed by clonal sequences of a second gene, to establish linkages between HIV-infected individuals with primary HIV infection [16]. However, linkage between individuals can never be established

with absolute certainty due to possible intermediary links, which may not have been sampled. Moreover, we cannot fully exclude that white and nonwhite individuals may have been infected abroad and later migrated to Switzerland together. However, the finding that 4 of the 26 possible transmission pairs were embedded in Swiss-specific subepidemics of size 3 ($n = 3$) or greater (size 14: $n = 1$; Figure 2) provides strong evidence that domestic infections with the 4 most prevalent non-B subtypes indeed do occur in Switzerland.

Overall, our results stand in contrast to findings of the subtype B epidemics among intravenous drug users (IDUs) and men who have sex with men (MSMs) in Switzerland, which were driven by domestic transmission [2], but also to the common notion that HIV infections with non-B subtypes are mainly a problem of migration. Instead, the epidemiology of non-B HIV-1 in Europe should be understood as the combined result of both migration and domestic transmission. Moreover, these findings have significant public health implications, because they demonstrate that less than one-fourth of all non-B infections diagnosed in Switzerland could possibly be prevented by local interventions, and they also suggest that awareness should be raised among immigrants and Swiss individuals with partners from countries with high HIV prevalence to contain the spread of HIV in Switzerland.

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A sample of 10% of the sequences used in this analysis were submitted to Genbank (Genbank Accession Numbers EF449787, EF449788, EF449796, EF449798, EF449828, EF449829, EF449838, EF449844, EF449852, EF449853, EF449854, EF449860, EF449880, EF449883, EF449889, EF449895, EF449901, EF449904, EF449905, EF449917, EF449921, EF449928, EF449930, EF449943, EF449950, EF449960, EF449971, EF449980, EF449987, EF450004, EF450005, EF450011, EF450024, EF450026, GQ848113, GQ848120, GQ848140, GQ848145, GQ848149, JF769777-JF769851].

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